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(54) LIPID CONTAINING 5,11,14-EICOSATRIENOIC ACID AND/OR 5,11,14,17-EICOSATETRAENOIC ACID AND METHOD FOR PRODUCING THE LIPID

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a method for the microbial production of 5,11,14-

eicosatrienoic acid and/or 5,11,14,17-eicosatetraenoic acid.

SOLUTION: The objective method for the production of 5,11,14-eicosatrienoic acid and/or 5,11,14,17-eicosatetraenoic acid or a triglyceride or lipid containing the fatty acids comprises the culture of a microorganism capable of producing arachidonic acid in a medium added with a $\Delta 6$ unsaturation reaction inhibitor and the collection of the produced lipid from the cultured product. The method enables the hitherto impossible industrial production of these fatty acids by microbial means.

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CLAIMS

[Claim(s)]

[Claim 1]A manufacturing method of 5 cultivating a microorganism which has arachidonic acid productivity by a culture medium which added delta6 desaturation reaction inhibitor, and extracting lipid from a culture, 11, and 14-eicosatrienoic acid content lipid.

[Claim 2]A manufacturing method of triglyceride containing 5 cultivating a microorganism which has arachidonic acid productivity by a culture medium which added delta6 desaturation reaction inhibitor, and extracting lipid from a culture, and carrying out separation refinement of the triglyceride from this lipid, 11, and 14-eicosatrienoic acid.

[Claim 3]A manufacturing method of 5 cultivating a microorganism which has arachidonic acid productivity by a culture medium which added delta6 desaturation reaction inhibitor, and extracting lipid from a culture, and carrying out separation refinement of 5, 11, and the 14-eicosatrienoic acid from this lipid, 11, and 14-eicosatrienoic acid.

[Claim 4]14-saturated fatty acid, oleic acid, linolic acid [or], 11, or eicosadienoic acid A microorganism which has arachidonic acid productivity. Or a manufacturing method given in any 1 paragraph of claims 1-3 cultivating these derivatives or fats and oils which contain these as a constituent by a culture medium added further.

[Claim 5]A manufacturing method given in any 1 paragraph of claims 1-3 cultivating a microorganism which has arachidonic acid productivity keeping a ratio of the linolic

acid/gamma-linolenic acid in a biomass or more at 30.

[Claim 6] A manufacturing method given in any 1 paragraph of claims 1–5 cultivating a microorganism which has arachidonic acid productivity at temperature of not less than 20 **.

[Claim 7] A ratio of the linolic acid/gamma-linolenic acid obtained from a microorganism which has arachidonic acid productivity cultivates a microorganism to which delta6 desaturation reaction which is 30 or more fell, And a manufacturing method of lipid which contains 5 extracting lipid from a culture, 11, and 14-eicosatrienoic acid 12% of the weight or more.

[Claim 8] A ratio of the linolic acid/gamma-linolenic acid obtained from a microorganism which has arachidonic acid productivity cultivates a microorganism to which delta6 desaturation reaction which is 30 or more fell, A manufacturing method of triglyceride containing 5 extracting lipid which contains 5, 11, and 14-eicosatrienoic acid 12% of the weight or more from a culture, and carrying out separation refinement of the triglyceride from this lipid, 11, and 14-eicosatrienoic acid.

[Claim 9] A ratio of the linolic acid/gamma-linolenic acid obtained from a microorganism which has arachidonic acid productivity cultivates a microorganism to which delta6 desaturation reaction which is 30 or more fell, A manufacturing method of 5 characterized by carrying out separation refinement of 5, 11, and the 14-eicosatrienoic acid from this lipid by extracting lipid which contains 5, 11, and 14-eicosatrienoic acid 12% of the weight or more from a culture, 11, and 14-eicosatrienoic acid.

[Claim 10] A manufacturing method of 5 characterized by extracting lipid from a culture by cultivating a microorganism which has arachidonic acid productivity at a temperature lower than 20 ** by a culture medium which added delta6 desaturation reaction inhibitor, 11, 14, and 17-eicosatetraenoic acid content lipid.

[Claim 11] A microorganism which has arachidonic acid productivity is cultivated at a temperature lower than 20 ** by a culture medium which added delta6 desaturation reaction inhibitor, A manufacturing method of 5 which extracts lipid from a culture and is characterized by carrying out separation refinement of the triglyceride from this lipid, 11, 14, and 17-eicosatetraenoic acid content triglyceride.

[Claim 12] A microorganism which has arachidonic acid productivity is cultivated at a temperature lower than 20 ** by a culture medium which added delta6 desaturation reaction inhibitor, A manufacturing method of 5 which extracts lipid from a culture and is characterized by carrying out separation refinement of 5, 11, 14, and the 17-eicosatetraenoic acid from this lipid, 11, 14, and 17-eicosatetraenoic acid.

[Claim 13] A microorganism which has arachidonic acid productivity Alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, or its derivative, Or a manufacturing method of 5 characterized by extracting lipid from a culture by cultivating by a culture

medium which added fats and oils which contain these as a constituent, and added delta6 desaturation reaction inhibitor further, 11, 14, and 17-eicosatetraenoic acid content lipid.

[Claim 14]A microorganism which has arachidonic acid productivity Alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, or its derivative, Or it cultivates by a culture medium which added fats and oils which contain these as a constituent, and added delta6 desaturation reaction inhibitor further, A manufacturing method of 5 which extracts lipid from a culture and is characterized by carrying out separation refinement of the triglyceride from this lipid, 11, 14, and 17-eicosatetraenoic acid content triglyceride.

[Claim 15]A microorganism which has arachidonic acid productivity Alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, or its derivative, Or it cultivates by a culture medium which added fats and oils which contain these as a constituent, and added delta6 desaturation reaction inhibitor further, A manufacturing method of 5 which extracts lipid from a culture and is characterized by carrying out separation refinement of 5, 11, 14, and the 17-eicosatetraenoic acid from this lipid, 11, 14, and 17-eicosatetraenoic acid.

[Claim 16]A microorganism which it has arachidonic acid productivity The Mortierella (Mortierella) group, A KONIDI obolus (Conidiobolus) group, a FICHIUMU (Pythium) group, A FITOFUTORA (Phytophthora) group, a PENISHIRYUMU (Penicillium) group, A KURADOSU volume (Cladosporium) group, the Mucor (Mucor) group, A FUZARYUMU (Fusarium) group, an Aspergillus (Aspergillus) group, The Rhodotorula (Rhodotorula) group, an ene TOMOFUTORA (Entomophthora) group, A manufacturing method given in any 1 paragraph of claims 1-15 being the microorganisms belonging to an EKINOSUPORANJUMU (Echinosporangium) group or a SAPUROREGUNIA (Saprolegnia) group.

[Claim 17]A manufacturing method given in any 1 paragraph of claims 1-16, wherein a microorganism which has arachidonic acid productivity is a microorganism belonging to the genus Mortierella subgenus Mortierella.

[Claim 18]A manufacturing method given in any 1 paragraph of claims 1-17 being the compounds in which the aforementioned delta6 desaturation reaction inhibitor has a methylenedioxyphenyl group.

[Claim 19]A compound with said methylenedioxyphenyl group A 3',4'-(methylenedioxy) acetophenone, The manufacturing method according to claim 18 being 3,4-(methylenedioxy) aniline, 1,2-(methylenedioxy)-4-nitrobenzene, piperonyl alcohol, piperonyl amine, PIPERONIRO nitril, or a safrole.

[Claim 20]A manufacturing method given in the any 1 paragraph according to claim 1 to 17, wherein the aforementioned delta6 desaturation reaction inhibitor is Parakou Mull acid ester.

[Claim 21]The manufacturing method according to claim 20, wherein said Parakou Mull acid ester is Parakou Mull acid methyl ester.

[Claim 22]A manufacturing method given in the any 1 paragraph according to claim 1 to 17, wherein the aforementioned delta6 desaturation reaction inhibitor is PARAA rock shell gin

(p-anisidine).

[Claim 23]Lipid which contains 5, 11, 5 whose a content of 14-eicosatrienoic acid is 15 % of the weight or more, 11, and 14-eicosatrienoic acid to total fatty acid in lipid.

[Claim 24]Lipid in which a content of 5, 11, and 14-eicosatrienoic acid contains 5 whose a content of 15 % of the weight or more, and 5, 11, 14 and 17-eicosatetraenoic acid is 0.1 or less % of the weight, 11, and 14-eicosatrienoic acid to total fatty acid in lipid.

[Claim 25]Triglyceride in which a content of 5, 11, and 14-eicosatrienoic acid contains 5 which is 12 % of the weight or more, 11, and 14-eicosatrienoic acid to total fatty acid in triglyceride.

[Claim 26]Triglyceride in which a content of 5, 11, and 14-eicosatrienoic acid contains 5 whose a content of 12 % of the weight or more, and 5, 11, 14 and 17-eicosatetraenoic acid is 0.1 or less % of the weight, 11, and 14-eicosatrienoic acid to total fatty acid in triglyceride.

[Claim 27]A food constituent which contains lipid or triglyceride of a statement in any 1 paragraph of claims 23-26.

[Claim 28]A cosmetic composition which contains lipid or triglyceride of a statement in any 1 paragraph of claims 23-26.

[Claim 29]A pharmaceutical composition which contains lipid or triglyceride of a statement in any 1 paragraph of claims 23-26.

[Claim 30]Feed for animals which contains lipid or triglyceride of a statement in any 1 paragraph of claims 23-26.

[Claim 31]delta6 desaturation reaction inhibitor of a microorganism which has the arachidonic acid productivity containing a compound with a methylenedioxyphenyl group.

[Claim 32]A 3',4'-(methylenedioxy) acetophenone, 3,4-(methylenedioxy) aniline, delta6 desaturation reaction inhibitor of a microorganism which has the arachidonic acid productivity containing either 1,2-(methylenedioxy)-4-nitrobenzene, piperonyl alcohol, piperonyl amine, PIPERONIRO nitril or a safrole.

[Claim 33]delta6 desaturation reaction inhibitor of a microorganism which has the arachidonic acid productivity containing Parakou Mull acid ester.

[Claim 34]delta6 desaturation reaction inhibitor of a microorganism which has the arachidonic acid productivity containing Parakou Mull acid methyl ester.

[Claim 35]delta6 desaturation reaction inhibitor of a microorganism which has the arachidonic acid productivity containing PARAA rock shell gin (p-anisidine).

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]In this invention, the microorganism which has arachidonic acid productivity is cultivated by the culture medium which added delta6 desaturation reaction inhibitor.

Therefore, 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, 17-eicosatetraenoic acid, Or 5 extracting the lipid which contains these as a constituent, 11, 14-eicosatrienoic acid and/or 5, 11, 14, 17-eicosatetraenoic acid, Or lipid or triglyceride containing the manufacturing method of the lipid which contains these as a constituent and 5, 11, 14-eicosatrienoic acid and/or 5, 11,14, and 17-eicosatetraenoic acid, It is related with the lipid which contains 5 and 11,14-eicosatrienoic acid 12% of the weight or more especially or triglyceride, and the constituent containing these.

[0002]

[Description of the Prior Art]5, 11, 14-eicosatrienoic acid, and 5, 11, 14 and 17-eicosatetraenoic acid are higher unsaturated fatty acids whose arrangement of a double bond is non-methylene intervention types.

It is thought that an operation of arachidonic acid and eicosapentaenoic acid (EPA) is rivaled, and the physiological function is expected.

Although a break through of these physiological functions is progressing now, It is thought that 5, 11, 14-eicosatrienoic acid, and 5, 11, 14 and 17-eicosatetraenoic acid have an operation different, respectively (Ikeda and others, Lipids, 27, 500-504, 1992.). Sugano Foodstuffs, development, 34, 4-8, 1999.

[0003]Although existing in a NAGI seed, an arbor vitae (biota) seed, a yew seed, a hard clam, starfish, etc. is known on the natural community, 5, 11, 14-eicosatrienoic acid, and 5, 11, 14 and 17-eicosatetraenoic acid, The amount of supply is scarce and unstable, and also these are expensive and are not practical. Moreover, there is a problem also in the point that the content of 5, 11, 14-eicosatrienoic acid, or 5, 11, 14 and 17-eicosatetraenoic acid is low, and the point which both are always contained and cannot control the presentation.

[0004]Free fatty acid containing 5 which disassembled and obtained the fats and oils of the arbor vitae (biota) seed as a method of condensing these fatty acid, 11, 14-eicosatrienoic acid, or 5, 11, 14 and 17-eicosatetraenoic acid, although the method of condensing using lipase of Candida origin is known (Jie MSFLK et al.) J. While there are Am. Oil Chem. Soc., 72, 245-249 (1995), and the above-mentioned problem too, there is a problem in that only free fatty acid is obtained, the field of cost, etc. Although the manufacturing method using the microorganism which has arachidonic acid productivity and to which delta6 desaturation activity fell as a manufacturing method by a microorganism is known (JP,5-276964,A), Since the degree to which delta6 desaturation activity is falling was not so remarkable, the

quantity of production and a content were low and were not necessarily practical.

[0005]About the compound which checks delta6 desaturation reaction. p-iso pentoxy aniline (p-isopentoxylaniline) has inhibitory action for an animal (Obukowicz MG et al., Biochem.Pharmacol., 55, and 1045–1058 (1998)), moreover — although SC-26196 and nifedipine (nifedipine) have inhibitory action in the in vitro experiment using a rat liver microsome fraction (Obukowicz MG et al.) J. Pharmacol. Exp. Ther., 287, and 157–166 (1998); [Kawashima et al.,] All of Biosci. Biotech. Biochem., 60, 1672–1676 (1996), and these compounds were [that the operation on the microsome fraction of an animal or an animal is only known, and].

[0006]about a microorganism, delta5 desaturation reaction and delta6 desaturation reaction can prevent simultaneously by adding propyl gallate (propyl gallate) in the case of culture of a genus Mortierella filamentous bacterium (Kawashima et al.) Biochim. Biophys. Acta, 1299, and 34–38 (1996) were known. However, accumulation of 5, 11, 14-eicosatrienoic acid, or 5, 11, 14 and 17-eicosatetraenoic acid was not accepted in this case. In order for 5, 11, and 14-eicosatrienoic acid to generate, linolic acid is chain length extension and delta 5. Although it is necessary to undergo two unsaturated reactions, It is delta 5 when propyl gallate (propylgallate) is added. In order to also check a desaturation reaction strongly, it is thought that 5, 11, and 14-eicosatrienoic acid did not generate.

[0007]Namely, delta6 desaturation reaction is specifically checked by adding to a culture medium in the case of the culture about a microorganism, The compound which, as a result, had in the biomass the operation in which 5, 11, 14-eicosatrienoic acid, and 5, 11, 14 and 17-eicosatetraenoic acid are stored up was not known. Thus, 5,11, 14-eicosatrienoic acid, and 5, 11, 14, 17-eicosatetraenoic acid, Or development of lipid with a high content of a manufacturing method with high productive efficiency of the lipid which contains these as a constituent and 5, 11, and 14-eicosatrienoic acid or triglyceride, and the constituent containing these is desired strongly.

[0008]

[Problem(s) to be Solved by the Invention]This invention Therefore, 5, 11, 14-eicosatrienoic acid and/or 5, 11,14, 17-eicosatetraenoic acid, Or it is going to provide the lipid which contains as a constituent the manufacturing method of the lipid which contains these as a constituent and 5, 11, and 14-eicosatrienoic acid or triglyceride, and the constituent containing these.

[0009]

[Means for Solving the Problem]This invention persons are [in / in order to attain the above-mentioned purpose, as a result of studying many things / culture of a microorganism] delta 5. A desaturation reaction was hardly affected but it found out that a compound which checks only delta6 desaturation reaction specifically existed. As such a compound, a 3',4'-(methylenedioxy) acetophenone, 3,4-(methylenedioxy) aniline,

1,2-(methylenedioxy)-4-nitrobenzene, A compound with methylenedioxyphenyl groups, such as piperonyl alcohol, piperonyl amine, PIPERONIRO nitril, and a safrole, and Parakou Mull acid ester and PARAA rock shell gin (p-anisidine) are mentioned.

[0010]By cultivating a microorganism which has arachidonic acid productivity by a culture medium which added these, Linolic acid which is a substrate of delta6 desaturation reaction is accumulated, and the linolic acid is changed into 11 and 14-eicosadienoic acid by a chain length extension-ized reaction, Generation accumulation of the lipid which this fatty acid is furthermore changed into 5, 11, and 14-eicosatrienoic acid by delta5 desaturation reaction, and contains this fatty acid is carried out into a biomass, [0011]By adding alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, its derivative, or fats and oils that contain these as a constituent to a culture medium in that case, Alpha-linoleic acid is changed into 11, 14, and 17-eicosatrienoic acid by a chain length extension-ized reaction, generation accumulation of the lipid which this fatty acid is furthermore changed into 5, 11, 14, and 17-eicosatetraenoic acid by delta5 desaturation reaction, and contains 5,11,14-eicosatrienoic acid and 5,11,14,17-eicosatetraenoic acid is carried out into a biomass — or [0012]By cultivating a microorganism which has arachidonic acid productivity by a culture medium which added a compound which checks only delta6 desaturation reaction specifically at a temperature lower than 20 **, 5 generated like the above-mentioned, 11, and 14-eicosatrienoic acid are further changed into 5, 11, 14, and 17-eicosatetraenoic acid by omega3 desaturation reaction, This invention persons found out that generation accumulation of the lipid containing 5, 11, 14-eicosatrienoic acid, and 5, 11, 14 and 17-eicosatetraenoic acid was carried out into a biomass, and this invention was completed.

[0013]Furthermore, this invention persons by continuing keeping a ratio of the linolic acid/gamma-linolenic acid in a biomass or more at 30, Conversion to 11 and 14-eicosadienoic acid from linolic acid had priority over conversion to dihome-gamma-linolenic acid from gamma-linolenic acid enough, and advanced, it found out that lipid which contains remarkable 5, 11, and 14-eicosatrienoic acid as the result generated, and this invention was completed.

[0014]Namely, this invention cultivates a microorganism which has arachidonic acid productivity by a culture medium which added delta6 desaturation reaction inhibitor, And a manufacturing method of 5 extracting lipid from a culture, 11, and 14-eicosatrienoic acid content lipid; a microorganism which has arachidonic acid productivity, A manufacturing method of triglyceride containing 5 cultivating by a culture medium which added delta6 desaturation reaction inhibitor, and extracting lipid from a culture, and carrying out separation refinement of the triglyceride from this lipid, 11, and 14-eicosatrienoic acid; it reaches. [0015]A microorganism which has arachidonic acid productivity is cultivated by a culture medium which added delta6 desaturation reaction inhibitor, A manufacturing method

of 5 extracting lipid from a culture and carrying out separation refinement of 5, 11, and the 14-eicosatrienoic acid from this lipid, 11, and 14-eicosatrienoic acid; in a row. In the aforementioned manufacturing method, a microorganism which has arachidonic acid productivity Saturated fatty acid, A manufacturing method cultivating 14-oleic acid, linolic acid, 11 or eicosadienoic acid, these derivatives, or fats and oils that contain these as a constituent by a culture medium added further is provided.

[0016] This invention again a microorganism which has arachidonic acid productivity Alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, or its derivative, Or a manufacturing method of 5 cultivating by a culture medium which added fats and oils which contain these as a constituent, and added delta6 desaturation reaction inhibitor further, and extracting lipid from a culture, 11, 14, and 17-eicosatetraenoic acid content lipid is provided. A manufacturing method of 5, wherein this invention cultivates again a microorganism which has arachidonic acid productivity at a temperature lower than 20 °C by a culture medium which added delta6 desaturation reaction inhibitor and extracts lipid from a culture, 11, 14, and 17-eicosatetraenoic acid content lipid is provided.

[0017]. [whether as for this invention, a microorganism which has arachidonic acid productivity is cultivated further, keeping a ratio of the linolic acid/gamma-linolenic acid in a biomass or more at 30, and] Or a ratio of the linolic acid/gamma-linolenic acid obtained from a microorganism which has arachidonic acid productivity cultivates a microorganism to which delta6 desaturation reaction which is 30 or more fell, And a manufacturing method of lipid which contains 5 extracting lipid from a culture, 11, and 14-eicosatrienoic acid 12% of the weight or more is provided.

[0018] Further this invention A 3',4'-(methylenedioxy) acetophenone, 3,4-(methylenedioxy) aniline, 1,2-(methylenedioxy)-4-nitrobenzene, A compound with methylenedioxyphenyl groups, such as piperonyl alcohol, piperonyl amine, PIPERONIROL nitril, and a safrole, delta6 desaturation reaction inhibitor of a microorganism which has the arachidonic acid productivity containing Parakou Mull acid ester and PARAA rock shell gin (p-anisidine) is provided.

[0019] Lipid in which this invention contains further 5, 11, 5 whose a content of 14-eicosatrienoic acid is 15 % of the weight or more, 11, and 14-eicosatrienoic acid to total fatty acid in lipid, And lipid in which a content of 5, 11, and 14-eicosatrienoic acid contains 5 whose a content of 15 % of the weight or more, and 5, 11, 14 and 17-eicosatetraenoic acid is 0.1 or less % of the weight, 11, and 14-eicosatrienoic acid to total fatty acid in lipid,

[0020] Triglyceride in which a content of 5, 11, and 14-eicosatrienoic acid contains 5 which is 12 % of the weight or more, 11, and 14-eicosatrienoic acid to total fatty acid in triglyceride, A content of 5, 11, and 14-eicosatrienoic acid to total fatty acid in triglyceride 12 % of the weight or more, And 5, 11, 14, 5 whose a content of 17-eicosatetraenoic acid is 0.1 or less % of the weight, 11, triglyceride containing 14-eicosatrienoic acid, and a constituent

containing the above-mentioned lipid or triglyceride are provided further.

[0021]

[Embodiment of the Invention] In this invention, if it is a microorganism which has arachidonic acid productivity, all can be used. As a microorganism which has arachidonic acid productivity, The *Mortierella* (*Mortierella*) group, a *KONIDI obolus* (*Conidiobolus*) group, A *FICHIUMU* (*Pythium*) group, a *FITOFUTORA* (*Phytophthora*) group, A *PENISHIRYUMU* (*Penicillium*) group, a *KURADOSU* volume (*Cladosporium*) group, The *Mucor* (*Mucor*) group, a *FUZARYUMU* (*Fusarium*) group, An *Aspergillus* (*Aspergillus*) group, the *Rhodotorula* (*Rhodotorula*) group, The microorganism belonging to an ene *TOMOFUTORA* (*Entomophthora*) group, an *EKINOSUPORANJIUMU* (*Echinosporangium*) group, and a *SAPUROREGUNIA* (*Saprolegnia*) group can be mentioned.

[0022] In the microorganism belonging to the *Mortierella* (*Mortierella*) group *Mortierella* (*Mortierella*) subgenuses. For example, *Mortierella* *ERONGATA* (*Mortierella elongata*), *Mortierella* *EKISHIGUA* (*Mortierella exigua*), the *Mortierella* *FIGURO* filler (*Mortierellahygrophila*), *Mortierella* *Alpina* (*Mortierella alpina*), etc. can be mentioned.

[0023] Specifically *Mortierella* *ERONGATA* (*Mortierella elongata*) IFO8570, *Mortierella* *EKISHIGUA* (*Mortierella exigua*) IFO8571, *Mortierella* *FIGURO* filler (*Mortierella hygrophila*) IFO5941, *Mortierella* *Alpina* (*Mortierella alpina*) IFO8568, ATCC16266, ATCC32221, and ATCC42430, The strain of CBS219.35, CBS224.37, CBS250.53, CBS343.66, CBS527.72, CBS529.72, CBS608.70, CBS754.68, etc. can be mentioned.

[0024] Each of these strains The foundation fermentation research institute (IFO) in Osaka, and American Type Culture Collection (American Type Culture Collection, ATCC) of the U.S. -- and, It can obtain from Centrralbureau voor Schimmelcultures (CBS) that there is no restriction in any way. Although strain *Mortierella* *ERONGATA* SAM0219 (FERM BP (Fermentation Research Institute mycoparasite No. 8703) No. 1239) which the research consortium of this invention separated from soil can also be used, it does not necessarily limit to these strains. Although the strain belonging to these type cultures or the strain separated from the nature can be used as it is, the spontaneous mutation stock with which character differs from the strain of the origin obtained by performing growth and/or isolation once or more can also be used.

[0025] In this invention, the microorganism to which delta6 desaturation activity acquired from the microorganism which has arachidonic acid productivity fell can be used. Namely, by cultivating the microorganism to which delta6 desaturation activity acquired from the microorganism which has arachidonic acid productivity fell by the culture medium which added delta6 desaturation reaction inhibitor, It becomes possible to raise the rate of the 5,11,14-eicosatrienoic acid in lipid or triglyceride, and/or 5,11,14,17- eicosatetraenoic acid.

[0026] When the ratio of the linolic acid/gamma-linolenic acid in a biomass uses the microorganism which is 30 or more also in the microorganism to which ** delta6

desaturation activity fell, Even if it does not add delta6 desaturation reaction inhibitor to a culture medium, the lipid which carries out remarkable content of 5, 11, and the 14-eicosatrienoic acid can be obtained. The microorganism to which such delta6 desaturation activity fell can be obtained by the mutation treatment indicated below, using the microorganism which has the above-mentioned arachidonic acid productivity. The activity of the enzyme which participates in delta6 desaturation reaction of the microorganism which has arachidonic acid productivity can be acquired also by a fall or the gene manipulation which carries out deletion. Although KO of the gene which encodes this enzyme, the inactivation by antisense, etc. are mentioned as the example, it does not necessarily limit to these methods.

[0027]Furthermore to this invention, 5, 11, 14-eicosatrienoic acid, and/. Or in order to raise the rate in the lipid of 5,11,14,17-eicosatetraenoic acid, or triglyceride, Use of the microorganism with which the chain length extension activity and delta5 desaturation activity which are acquired by performing mutation treatment and gene manipulation were strengthened is also included to the microorganism to which delta6 desaturation activity acquired from the microorganism which has the above-mentioned arachidonic acid productivity, or this microorganism fell.

[0028]In this invention, mutation treatment A radiation (X-rays, gamma ray, neutron beam) exposure and UV irradiation, Mutagen is added, after fixed time incubation, it can dilute suitably, inoculation can be carried out [high temperature processing etc. can be performed, and a microorganism can be suspended in a suitable buffer etc.,] to an agar medium, and general mutation operation of obtaining the colony of a variant can also be performed.

[0029]As mutagen, they are nitrogen mustard, and a methylmethane sulfonate and the N-methyl-N'-nitro N. - To nitrosoguanidine (NTG) etc., alkylating agent, Coloring matter, such as base synthesis inhibitor, such as antibiotics, such as base analogs, such as 5-bromouracil, and mitomycin-C, and 6-mercaptopurine, and proflavine, 4-nitroquinoline N - Compounds, such as a carcinogenic agent of a certain kind, such as oxide, a manganese chloride, and formaldehyde, can be mentioned. The colony obtained by said mutation operation analyzes the fatty acid composition in a biomass in accordance with a general method, and chooses the bacillus in which delta6 desaturation reaction fell.

[0030]For example, although strain *Mortierella Alpina* SAM1969 (the 12901st item of the Fermentation Research Institute mycoparasite) to which delta6 desaturation activity from which the research consortium of this invention performed and got mutation processing fell can also be used as a variant of this invention, it does not necessarily limit to these strains. In order to cultivate the strain used for this invention, the spore, the fungal thread, or the preculture liquid produced by cultivating beforehand of the strain is inoculated into a liquid medium or a solid medium, and is cultivated. In the case of a liquid medium, as a carbon source, each thing generally used, such as glucose, fructose, xylose, saccharose, malt sugar,

soluble starch, molasses, glycerol, and mannitol, can use it, but it is not restricted to these. [0031]As a nitrogen source, peptone, a yeast extract, a malt extract, a meat extract, casamino acids, Sources of inorganic nitrogen other than natural nitrogen sources, such as corn steep liquor, soybean protein, degreasing soybeans, and cottonseed dregs, such as organic nitrogen sources, such as urea, and sodium nitrate, ammonium nitrate, and ammonium sulfate, can be used. In addition, mineral salt, a vitamin, etc. of an phosphate, magnesium sulfate, ferrous sulfate, copper sulfate, etc. can be used as a source of micronutrient if needed.

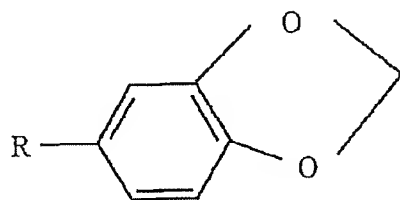
[0032]If these medium components are concentration which does not injure growth of a microorganism, there will be no restriction in particular. practically — general — a carbon source — 0.1 to 40 % of the weight [] — desirable — 1 to 25 % of the weight It is good to consider it as concentration. Feeding of the carbon source may be carried out in the middle of culture one by one. the nitrogen source addition of initiation — 0.1 to 10 % of the weight [] — desirable — 0.1 to 6 % of the weight It may carry out and feeding of the nitrogen source may be carried out in the middle of culture.

[0033]All the compounds that check delta6 desaturation reaction of the microorganism to be used specifically and in which 5, 11, 14-eicosatrienoic acid, and 5, 11 and 14,17-eicosatetraenoic acid are stored up can be used for delta6 desaturation reaction inhibitor used for this invention. delta5 desaturation reaction which changes 11 and 14-eicosadienoic acid into 5, 11, and 14-eicosatrienoic acid preferably, And in order to advance delta5 desaturation reaction which changes 11, 14, and 17-eicosatrienoic acid into 5, 11, 14, and 17-eicosatetraenoic acid, the one where delta5 desaturation reaction inhibition operation is smaller is good. As such a compound, the following compound can be mentioned, for example.

[0034]

[Formula 1]

メチレンジオキシフェニル基を有する化合物

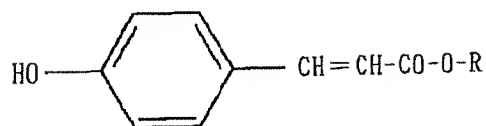


Rが $-CO-CH_3$, $-NH_2$, $-NO_2$, $-CH_2-OH$, $-CH_2-NH_2$, $-CN$ 又は $-CH_2-CH=CH_2$

[0035]

[Formula 2]

パラクマル酸エステル

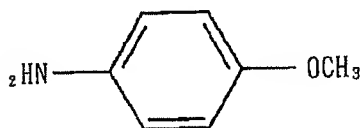


Rは低級アルキル基

[0036]

[Formula 3]

パラアニシジン (p-anisidine)



[0037]As a compound with a methylenedioxyphenyl group, 3 -- ' -- 4'-(methylenedioxy) acetophenone and 3,4-(methylenedioxy) aniline. 1,2-(methylenedioxy)-4-nitrobenzene, piperonyl alcohol, piperonyl amine, PIPERONIRO nitril, and a safrole are preferred, and Parakou Mull acid methyl ester is preferred as Parakou Mull acid ester. If the total addition of delta6 desaturation reaction inhibitor is concentration which does not injure growth of a microorganism, there will be no restriction in particular. receiving a culture medium generally practically -- 0.0001 to 10 % of the weight [] -- desirable -- 0.01 to 10 % of the weight it is .

[0038]After it may add the front stirrup into which these delta6 desaturation reaction inhibitor inoculates a microorganism immediately after that or it starts culture, it may be added, or it may be added at both the times. The addition after a culture start is 1. A time may be sufficient, or it may divide into multiple times and may add intermittently. Or it can also add continuously. delta6 desaturation reaction inhibitor may be used alone and may be used combining two or more compounds.

[0039]a microorganism which uses culture temperature of a microorganism of this invention -- a twist -- alias -- although it becomes, 5-40 ** shall be 10-30 ** preferably. culture temperature in a case of making lipid which does not contain 5, 11, 14, and 17-eicosatetraenoic acid substantially, but contains 5, 11, and 14-eicosatrienoic acid produce -- temperature of not less than 20 ** -- 20-40 ** shall be 20-30 ** more preferably. If not contained substantially, 5, 11, 14, and 17-eicosatetraenoic acid mean 0.1 or less % of the weight of a case to total fatty acid in lipid.

[0040]However, alpha-linoleic acid which is a precursor of 5, 11, 14, and 17-eicosatetraenoic acid at a culture medium even when cultivating at this temperature or its derivative and/or 11 and 14, 17-eicosatrienoic acid, or its derivative, Or when fats and oils which contain these as a constituent are added and cultivated, 5, 11, and 14,17-eicosatetraenoic acid are also produced.

[0041]Alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, or its derivative, Or culture temperature in a case of making 5, 11, 14, and 17-eicosatetraenoic acid produce without adding fats and oils which contain these as a constituent to a culture medium, It is considered as a temperature lower than 20 **, a desirable temperature lower than not less than 5 ** 20 **, and a more desirable temperature lower than not less than 10 ** 20 **. pH of a culture medium -- 4-10 -- aeration spinner culture, shaking culture, or standing culture is preferably performed as 5-9. culture -- usually -- two to 30 days -- it carries out for five to 15 days more preferably for five to 20 days.

[0042]bran which added water of 50-100 weight % to drained weight when cultivating by solid culture -- it rubs and culture is performed for three to 14 days at 5-40 ** and the desirable aforementioned temperature using ** et al., rice bran, etc. In this case, a nitrogen source, mineral, and a source of micronutrient can be added into a culture medium if needed.

[0043]In this invention, as a precursor of 5, 11, and 14-eicosatrienoic acid, saturated fatty acid, Production of 5, 11, and 14-eicosatrienoic acid can be made to increase by adding 14-oleic acid, linolic acid, 11 or eicosadienoic acid, these derivatives, or fats and oils that contain these as a constituent to a culture medium. As a precursor of 5, 11, 14, and 17-eicosatetraenoic acid, alpha-linoleic acid or its derivative and/or 11, 14, 17-eicosatrienoic acid, or its derivative, Or 5, 11, 14, and 17-eicosatetraenoic acid can be made to produce by adding fats and oils which contain these as a constituent to a culture medium.

[0044]in each case, the total addition of a precursor receives at a culture medium -- 0.001 to 10 % of the weight [] -- desirable -- 0.5 to 10 % of the weight it is . After it may add a front stirrup into which these precursors inoculate a production microorganism immediately after that or it starts culture, it may be added, or it may be added at both the times. Addition after a culture start is 1. A time may be sufficient, or it may divide into multiple times and may add intermittently. Or it can also add continuously.

[0045]It is concerned with existence of production of 5, 11, 14, and 17-eicosatetraenoic acid, there is nothing, and the substrate can be added in order to raise a generated amount of 5, 11, and 14-eicosatrienoic acid. As a substrate, hydrocarbon, such as tetradecane, hexadecane, and octadecane, Although fatty acid, such as tetradecanoic acid, hexadecanoic acid, and octadecanoic acid, or salts (for example, sodium salt, potassium salt, etc.) of those and ester, or fatty acid can mention fats and oils (for example, olive oil, safflower oil, palm oil) etc. which are contained as a constituent, It is not restricted to these.

[0046]The total addition of a substrate is 0.001 to 10 % of the weight to a culture medium. It is 0.5 to 10 % of the weight preferably. After it may add a front stirrup into which these substrates inoculate a production microorganism immediately after that or it starts culture, it may be added, or it may be added at both the times. Addition after a culture start is 1. A time may be sufficient, or it may divide into multiple times and may add intermittently. Or it can also add continuously.

[0047]In order to obtain lipid or triglyceride containing 5,11,14-eicosatrienoic acid and/or 5,11,14,17- eicosatetraenoic acid with yield which can be commercialized, Using a liquid medium, ventilation stirring culture is preferred and can also use a usual churning type fermenter or a bubbling tower type culture apparatus. As quantity of airflow, 10 – 500 rpm has desirable 0.1 – 3 vvm as an agitating speed.

[0048]It cultivates in this way and generation accumulation of the lipid which contains 5,11,14-eicosatrienoic acid and/or 5,11,14,17- eicosatetraenoic acid in a biomass is carried out. Culture medium or its sterilized culture medium in the middle of manufacturing lipid by biomass culture, when a liquid medium is used, Or lipid which contains 5,11,14-eicosatrienoic acid and/or 5,11,14,17- eicosatetraenoic acid from a culture object which carried out the harvest from culture medium at the time of an end of culture, or the sterilized culture medium or each, or its dry matter is extracted. For example, extraction of lipid which contains 5,11,14-eicosatrienoic acid and/or 5,11,14,17- eicosatetraenoic acid as follows from a culture object, And isolation of triglyceride containing 5,11,14-eicosatrienoic acid and/or 5,11,14,17- eicosatetraenoic acid or 5,11,14-eicosatrienoic acid, and/or 5,11,14,17- eicosatetraenoic acid is performed.

[0049]A culture object is acquired by a solid liquid separation means of daily use, such as a centrifuge method and filtration, from culture medium after an end of culture. A biomass is rinsed enough and dried preferably. Freeze-drying, air-drying, etc. can perform desiccation. After crushing a dried cell, for example with dynamill, an ultrasonic wave, etc., extracting processing of it is preferably carried out with an organic solvent under a nitrogen air current. As an organic solvent, ether, hexane, methanol, ethanol, chloroform, Dichloromethane, petroleum ether, etc. can be used and it is mutual extraction and chloroform of methanol and petroleum ether. Methanol A good result can be obtained also by extraction of water using a solvent of a system much more. By distilling an organic solvent out of an extract under decompression, lipid containing 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid is obtained.

[0050]It can replace with an above-mentioned method and can extract using a wet fungus body. In this case, a mixed solvent of compatibility is used to water which consists of a solvent of compatibility or these and water, and/or other solvents to water, such as methanol and ethanol. Other procedures are the same as that of the above.

[0051]In lipid produced by performing it above, 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14,

and 17-eicosatetraenoic acid, Neutral lipid, such as triglyceride, diglyceride, monoglyceride, and sterol ester, Phosphatidylcholine, a lysophosphatidylcholine, a phosphatidylethanolamine, A lysophosphatidylethanolamine, a phosphatidylinositol, It exists as a constituent of polar lipid, such as lysophosphatidylinositol, a phosphatidyl serine, lysophosphatidylserine, phosphatidic acid, and a lysophosphatidic acid, or free fatty acid.

[0052]For example, as a fats-and-oils presentation of lipid containing 5 manufactured using a microorganism belonging to the subgenus Mortierella, 11,14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid, Triglyceride which neutral lipid is 70 to 100 % of the weight, and polar lipid is 0 to 30 % of the weight, and is the main ingredients of neutral lipid is 70 to 99 % of the weight.

[0053]In the case of lipid which does not contain 5, 11, 14, and 17-eicosatetraenoic acid substantially, 5 in lipid, 11, and 14-eicosatrienoic acid content, It is 2.5 to 80 % of the weight more preferably 1.3 to 80% of the weight 0.2 to 80% of the weight to total fatty acid. In the case of lipid containing 5, 11, 14, and 17-eicosatetraenoic acid, 5 in lipid, 11, 14, and 17-eicosatetraenoic acid content, To total fatty acid, 0.1 to 70% of the weight, preferably, although it is 0.5 to 70 % of the weight more preferably, this 5, 11, 14, and 17-eicosatetraenoic acid content change with a kind of that precursor, an addition, or culture temperature 0.2 to 70% of the weight.

[0054]A rate over total fatty acid in triglyceride in lipid, In the case of lipid which does not contain 5, 11, 14, and 17-eicosatetraenoic acid substantially. 5, 11, and 14-eicosatrienoic acid 3.0 to 80 % of the weight, It is 13.4 to 80 % of the weight more preferably 12.1 to 80% of the weight, In the case of lipid containing 5, 11, 14, and 17-eicosatetraenoic acid, 5 in lipid, 11, 14, and 17-eicosatetraenoic acid content, To total fatty acid, 0.1 to 70% of the weight, preferably, although it is 0.5 to 70 % of the weight more preferably, 0.4 to 70% of the weight, This 5, 11, 14, and 17-eicosatetraenoic acid content can be freely changed by adjusting a kind of that precursor, an addition, or *****.

[0055]In this invention, lipid which contains 5, 11, 5 whose a content of 14-eicosatrienoic acid is 15 % of the weight or more, 11, and 14-eicosatrienoic acid to total fatty acid in lipid can be obtained. A content of 5, 11, and 14-eicosatrienoic acid can obtain lipid containing 5 whose a content of 15 % of the weight or more, and 5, 11, 14 and 17-eicosatetraenoic acid is 0.1 or less % of the weight, 11, and 14-eicosatrienoic acid to total fatty acid in lipid.

[0056]As for the above-mentioned lipid, higher fatty acid says a certain chemical bond and a substance meltable to organic solvents, such as alcohol, chloroform, and benzene, insoluble to water and which formed an ester bond typically by the intramolecular. 5, 11, 14-eicosatrienoic acid, and/. As an example of contained lipid, 5, 11, 14, and 17-eicosatetraenoic acid Or 5, 11, 14-eicosatrienoic acid, and/. Or lower alkyl ester of 5, 11, and 14,17-eicosatetraenoic acid, 5, 11, 14-eicosatrienoic acid, and/. Or glycerol ester which contains 5, 11, and 14,17-eicosatetraenoic acid as a constituent or sterol ester, two or

more sorts of arbitrary mixtures of these, etc. are mentioned.

[0057]The above 5, 11, 14-eicosatrienoic acid, and/. With or lower alkyl ester of 5, 11, 14, and 17-eicosatetraenoic acid. 1-6 carbon numbers say 1-4 ester of 1-3 lower alcohol, 5, 11 and 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid more preferably. 5, 11, 14-eicosatrienoic acid, and/. With or glycerol ester which contains 5, 11, 14, and 17-eicosatetraenoic acid as a constituent. A substance in which 5 [at least one-molecule], 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid carried out the ester bond is said to one molecule of glycerin.

[0058]As the example, triglyceride, diglyceride, monoglyceride, phosphatidylcholine, A lysophosphatidylcholine, a phosphatidylethanolamine, A lysophosphatidylethanolamine, a phosphatidylinositol, lysophosphatidylinositol, a phosphatidyl serine, lysophosphatidylserine, phosphatidic acid, a lysophosphatidic acid, glyceroglycolipid, etc. are mentioned.

[0059]5, 11, 14-eicosatrienoic acid, and/. Or sterol ester which contains 5, 11, and 14,17-eicosatetraenoic acid as a constituent means a substance in which sterol, 5, 11 and 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid carried out the ester bond. A cholesterol ester, desmosterol ester, etc. are mentioned as the example. 5, 11, 14-eicosatrienoic acid, and/. Or lipid containing 5, 11, 14, and 17-eicosatetraenoic acid is not limited to the above example, but contains arbitrary lipid included by the above-mentioned definition, such as sphingophospholipid, other phospholipid, ceramide, sphingoglycolipid, and other glycolipids.

[0060]Furthermore in this invention, total fatty acid in triglyceride in lipid is received, Triglyceride in which a content of 5, 11, and 14-eicosatrienoic acid contains preferably 5 which is 14 % of the weight or more more preferably, 11, and 14-eicosatrienoic acid 13% of the weight or more 12% of the weight or more can be obtained. In a content of 5, 11, and 14-eicosatrienoic acid, a content of 12 % of the weight or more, and 5, 11 and 14,17-eicosatetraenoic acid to total fatty acid in triglyceride in lipid 0.1 or less % of the weight, A content of 13 % of the weight or more, and 5, 11, 14 and 17-eicosatetraenoic acid preferably 0.1 or less % of the weight, Triglyceride in which a content of 14 % of the weight or more, and 5, 11, 14 and 17-eicosatetraenoic acid contains more preferably 5 which is 0.1 or less % of the weight, 11, and 14-eicosatrienoic acid can be obtained.

[0061]From lipid containing 5 extracted from a culture, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid. In order to carry out separation refinement of the triglyceride containing 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid, In accordance with a conventional method, it carries out by the deoxidizing method, odor treatment, degumming method, the drying method, steam distillation method, molecular distillation method, cooling separation method, the column chromatography method, etc., for example. For example, lipid which contains 5,11,14-eicosatrienoic acid and/or 5,11,14,17-eicosatetraenoic acid using hexane according

to the above-mentioned operation from a culture is extracted, Triglyceride of this invention can be obtained from this extracted oil by purification treatment, such as deoxidation, deodorization, and a degumming.

[0062]In order to separate 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid from lipid containing 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid, In the state of mixed fatty acid or mixed fatty acid ester, it carries out with a conventional method by carrying out concentration separation by urea adduct method, cooling separation method, the column chromatography method, etc., for example.

[0063]Although 5, 11, 14-eicosatrienoic acid and/or 5, 11,14, and 17-eicosatetraenoic acid can also more specifically be separated directly, It is preferred to dissociate as ethyl ester of ester with lower alcohol, for example, 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid. By using such ester, are easily separable from other lipid components, It is easily separable from other fatty acid generated during culture, for example, pulmitic acid etc., (these are also esterified when esterifying 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid).

[0064]For example, in order to obtain ethyl ester of a higher unsaturated fatty acid, it is dehydrated ethanol about the aforementioned extraction lipid. It is preferred to carry out 1-24 time processing at a room temperature by 5 to 10% of chloride, 10 to 50% of BF_3 -ethanol, etc. For collecting ethyl ester of 5,11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid from the aforementioned treating solution, it is preferred that organic solvents, such as hexane, ether, and ethyl acetate, extract. Next, this extract is dried with anhydrous sodium sulfate etc., and a mixture which contains fatty acid ester as the main ingredients is obtained by distilling off an organic solvent under decompression preferably. This mixture can raise concentration of fatty acid of this invention as it is or further, and can use it for a constituent of this invention.

[0065]Fatty acid ethyl ester other than ethyl ester of 5 made into the purpose, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid, such as pulmitic acid ethyl ester, is contained in this mixture. In order to isolate ethyl ester of 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid from these fatty-acid-ethyl-ester mixtures, It is independent, or column chromatography, the low-temperature crystallizing method, a urea inclusion method, *** exchange partitionary chromatography, etc. can be combined and used.

[0066]It carries out like this. In order to obtain 5 of isolation, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid from ethyl ester of 5 which isolated, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic acid, What is necessary is for organic solvents, such as ether and ethyl acetate, just to extract, after alkali hydrolyzes. In order to take in 5, 11, 14-eicosatrienoic acid and/or 5, 11, 14, and 17-eicosatetraenoic

acid without passing through the ethyl ester, After carrying out alkaline degradation (it is 2 to 3 hours at a room temperature for example, by 5% sodium hydroxide) of the aforementioned extraction lipid, it can extract and refine by a method regularly used by extraction and refining of fatty acid from this decomposition liquid. Fatty acid of this invention of obtained isolation and its salt can also be used for a constituent of this invention.

[0067]Lipid or triglyceride containing 5 of this invention, 11, 14-eicosatrienoic acid and/or 5, 11, and 14,17-eicosatetraenoic acid, It comes out for there to be an infinite possibility about the use and to use it with lipid or triglyceride which contains this fatty acid abundantly, as a raw material and additives, such as foodstuffs, a drink, cosmetics, drugs, and feed for animals. For example, although common foodstuffs, a drink, functional food, a supplement, modified milk, face toilet, a milky lotion, an enteral hyperalimentation drug, powder, granulation, troches, syrup, a tablet, a capsule, an infusion solution, injections, gel for spreading, a packing sheet, powder feed, a pellet, liquefied feed, etc. can be mentioned, It does not limit to these and restriction is not received at all about the purpose of use, the amount used, and a processing form.

[0068]

[Example]Next, an example explains this invention still more concretely. However, this invention does not receive limitation in an example.

5 using example 1. delta6 desaturation reaction inhibitor, 11, 14-eicosatrienoic acid, One platinum loop of 5, 11, 14, and Mortierella Alpina (Mortierella alpina) IFO8568 that have the manufacturing method arachidonic acid productivity of 17-eicosatetraenoic acid and that are microorganisms, Liquid-medium 2mL put into 10mL Erlenmeyer flask (glucose 4%, inoculation was carried out to 1% of a yeast extract, and the following delta6 desaturation reaction inhibitor (concentration shown in Table 1), and shaking culture was carried out for ten days at 28 °C and 120 rpm.)

[0069]Biomasses were collected by filtration after culture and it dried. After carrying out methyl esterification of the fatty acid in a biomass with chloride methanol in accordance with a conventional method, it extracted by hexane and gas chromatography analyzed the fatty acid methyl ester produced by distilling off hexane. A result is shown in Table 1.

[0070](1) 3', 4'- (Methylenedioxy) An acetophenone. (2) 3,4-(methylenedioxy) aniline (3) 1,2-(methylenedioxy)-4-nitrobenzene (4) Piperonyl alcoholic (5) piperonyl-amine (6) PIPERONIRO nitril (7) safrole (8) Parakou Mull acid methyl ester (9) PARAA rock shell gin

[0071]

[Table 1]

表 1

△ 6 不飽和化反応阻害剤による脂肪酸組成の変化

△ 6 不飽和化 反応阻害剤		総脂肪酸に対する割合 (%)									
種類	濃度 (%)	16:0	18:0	18:1	18:2	18:3	8TA	ETeA	20:3	20:4	その他
(1)	0.02	16.7	9.6	22.5	26.0	1.7	2.5	0.0	2.0	15.2	3.8
(2)	0.02	12.2	9.7	8.5	16.3	2.1	0.2	0.0	4.0	45.5	1.5
(3)	0.02	17.6	18.8	12.3	23.9	1.5	0.2	0.0	3.5	20.4	1.8
(4)	0.02	18.3	9.1	13.5	17.0	1.9	0.2	0.0	4.1	34.7	1.2
(5)	0.02	15.2	10.3	13.2	32.8	1.5	6.3	0.0	2.2	13.4	5.1
(6)	0.02	19.7	9.3	23.0	17.7	1.0	0.2	0.0	3.9	23.1	2.1
(7)	0.05	19.0	10.1	12.1	34.5	1.0	5.6	0.0	1.9	13.7	2.1
(8)	0.02	17.6	9.9	13.2	28.0	1.8	3.1	0.0	3.5	19.7	3.2
(9)	0.1	19.8	10.5	12.2	32.8	1.3	4.8	0.0	2.3	15.9	0.4
なし	—	13.4	7.1	7.8	6.3	4.5	0.0	0.0	5.8	54.7	0.4

16:0、パルミチン酸；18:0、ステアリン酸；18:1、オレイン酸；18:2、リノール酸；18:3、
γ-リノレン酸；8TA、5, 11, 14-エイコサトリエン酸；ETeA、5, 11, 14, 17-エイ
コサテトラエン酸；20:3、ジホモγ-リノレン酸；20:4、アラキドン酸

[0072]It turned out that any delta6 desaturation reaction inhibitor stores up linolic acid, and 5, 11 and 14-eicosatrienoic acid. As for neither, 5, 11, 14, and 17-eicosatetraenoic acid were detected.

[0073]One platinum loop of a microorganism which has the arachidonic acid productivity of the manufacturing method following of 5 by the microorganism which has example 2. arachidonic acid productivity, 11, and 14-eicosatrienoic acid. Inoculation was carried out to liquid-medium 2mL (glucose 4%, 1% of a yeast extract, piperonyl amine 0.05 %) put into 10mL Erlenmeyer flask, and shaking culture was carried out for ten days at 28 ** and 120 rpm. Gas chromatography analyzed the fatty acid methyl ester obtained by carrying out methyl esterification like Example 1 after culture. A result is shown in Table 2.

[0074](1) *Mortierella Alpina* (*Mortierella alpina*) IFO8568(2) *Mortierella Alpina* (*Mortierella alpina*) SAM1969 (the 12901st item of the Fermentation Research Institute mycoparasite) (3) The *Mortierella* FIGURO filler. (*Mortierella hygrophila*) IFO5941(4) *KONIDI obolus* slow BOIDESU (*Conidiobolus thromboides*) CBS183.60(5) *FICHIUMU IREGURARE* (*Pythium.*) *irregulare* CBS494.86 (6) *FITOFUTORA yne festival wardrobe* (*Phytophthora infestans*) IFO4872(7) *EKINOSUPORANJUMU* Torrance Bell Sale (*Echinosporangium.*) *transversale* NRRL3116 (8) *SAPUOREGUNIARAPONIKA* (*Saprolegnia lapponica*) CBS313.81 [0075]

[Table 2]

表 2

アラキドン酸生産能を有する微生物による 5, 11, 14-エイ
コサトリエン酸の生産

菌株	ピペロニル アミン (%)	5, 11, 14-エイコサトリエン酸	
		生成量 (mg/培地1L)	総脂肪酸量に 対する割合 (%)
(1)	0 0.05	0 78	0 3.4
(2)	0 0.05	24 209	1.3 12.0
(3)	0 0.05	0 34	0 2.2
(4)	0 0.05	0 18	0 1.2
(5)	0 0.05	0 28	0 2.0
(6)	0 0.05	0 18	0 1.1
(7)	0 0.05	0 50	0 2.4
(8)	0 0.05	0 22	0 1.8

[0076]When any strain added piperonyl amine, generation of 5, 11, and 14-eicosatrienoic acid was accepted. Also in *Mortierella Alpina* (*Mortierella alpina*) SAM1969 (the 12901st item of the Fermentation Research Institute mycoparasite) in which not adding piperonyl amine also generates 5, 11, and 14-eicosatrienoic acid, By adding, the rate over a generated amount and the amount of total fatty acid increased remarkably. As for neither of the cases, 5, 11, 14, and 17-eicosatetraenoic acid were detected.

[0077]5 by example 3. low-temperature culture, 11, and 14, manufacturing method *Mortierella Alpina* (*Mortierella alpina*) IFO8568 of 17-eicosatetraenoic acid, or *Mortierella Alpina* (*Mortierella alpina*) SAM1969 (the [Fermentation Research Institute mycoparasite] -- 12901.) Inoculation of the one platinum loop of an item was carried out to liquid-medium 2mL (glucose 4%, 1% of a yeast extract, piperonyl amine 0.05 %) put into 10mL Erlenmeyer flask, and shaking culture was carried out for ten days at 120 rpm at 12-28 **. Gas chromatography analyzed the fatty acid methyl ester obtained by carrying out methyl esterification like Example 1 after culture. A result is shown in Table 3.

[0078]

[Table 3]

表 3

低温培養における5, 11, 14, 17-エイコサテトラエン酸の生産

生産菌株	温度 (℃)	5, 11, 14-エイコサトリエン酸		5, 11, 14, 17-エイコサテトラエン酸	
		生成量 (mg/培地1L)	総脂肪酸量に 対する割合 (%)	生成量 (mg/培地1L)	総脂肪酸量に 対する割合 (%)
モルティエラ アルピナ IFO8568	28	78	3.4	0	0
	20	77	3.3	3	0.1
	12	62	3.5	11	0.6
モルティエラ アルピナ SAM1969	28	212	12.5	0	0
	20	239	12.0	2	0.1
	12	194	11.0	34	1.9

Any strain generated 5, 11, 14, and 17-eicosatetraenoic acid, only when it cultivated below 20 **.

[0079]Example 4, alpha-linoleic acid, 11, 14, 17-eicosatrienoic acid, Or one platinum loop of 5 by addition of these related compounds, 11, 14, and manufacturing method Mortierella Alpina (Mortierella alpina) IFO8568 of 17-eicosatetraenoic acid, Inoculation was carried out to liquid-medium 2mL (the compound shown in Table 4 is added 0.5% glucose 4% to 1% of a yeast extract, and the culture medium which consists of piperonyl amine 0.05 %) put into 10mL Erlenmeyer flask, and shaking culture was carried out for ten days at 28 ** and 120 rpm.

[0080]Gas chromatography analyzed the fatty acid methyl ester obtained by carrying out methyl esterification of a part of biomass like Example 1 after culture. The remaining biomass performed chloroform methanol extraction after desiccation. Furthermore in accordance with the conventional method, fractionation of the extracted oil was carried out by thin layer chromatography and silicic acid column chromatography, and triglyceride was obtained. Also about the obtained triglyceride, it analyzed with gas chromatography by the above-mentioned method. A result is shown in Table 4.

[0081]

[Table 4]

表4

前駆体添加による5, 11, 14, 17-エイコサテトラエン酸の生産

添加する化合物	5, 11, 14, 17-エイコサテトラエン酸		
	生成量 (mg/培地1L)	菌体脂質中の 総脂肪酸量に 対する割合 (%)	トリグリセリ ド中の総脂肪 酸量に対する 割合 (%)
α -リノレン酸	10	0.5	0.4
α -リノレン酸メチル	32	1.6	1.4
α -リノレン酸エチル	30	1.4	1.2
α -リノレン酸ナトリウム	10	0.6	0.5
11, 14, 17-エイコサトリエン酸	16	0.8	0.7
11, 14, 17-エイコサトリエン酸 メチル	40	2.1	1.9
大豆油	5	0.2	0.1
アマニ油	35	1.3	1.1
なし	0	0	0

[0082] By adding further α -linoleic acid or its derivative, 11, 14, 17-eicosatrienoic acid, its derivative, or the fats and oils that contain these as a constituent to the culture medium containing piperonyl amine, The lipid containing 5, 11, 14, and 17-eicosatetraenoic acid generated. Triglyceride containing 5,11,14,17-eicosatetraenoic acid was contained in the generated lipid.

[0083] One platinum loop of Example 5.5, 11, and addition effect Mortierella Alpina (Mortierella alpina) IFO8568 of the substrate exerted on 14-eicosatrienoic acid production, Inoculation was carried out to liquid-medium 2mL (glucose 4%, 1% of a yeast extract, piperonyl amine the compound shown in Table 5 is added 0.5% to the culture medium which consists of 0.05%) put into 10mL Erlenmeyer flask, and shaking culture was carried out for ten days at 28 °C and 120 rpm. Gas chromatography analyzed the fatty acid methyl ester obtained by carrying out methyl esterification like Example 1 after culture. A result is shown in Table 5.

[0084]

[Table 5]

表 5

基質添加による 5, 11, 14-エイコサトリエン酸の生産

添加する基質	5, 11, 14-エイコサトリエン酸 生成量 (mg/培地1L)
テトラデカン	81
ヘキサデカン	91
オクタデカン	90
ヘキサデカン酸	90
ヘキサデカン酸メチル	95
ヘキサデカン酸ナトリウム	89
オリーブ油	92
サフラワー油	90
なし	70

Any substrate was effective in making the generated amount of 5, 11, and 14-eicosatrienoic acid increase.

[0085] One platinum loop of 5 using example 6.10L ***** culture, 11, and mass culture method *Mortierella Alpina* (*Mortierella alpina*) SAM1969 (the 12901st item of the Fermentation Research Institute mycoparasite) of 14-eicosatrienoic acid, Inoculation was carried out to liquid-medium 100mL (glucose 2 %, 1% of yeast extract) put into the 500mL Erlenmeyer flask, and preculture was carried out for seven days at 120 rpm at 28 **. The culture medium (glucose 2 %, 1% of a yeast extract, piperonyl amine 0.05%) of 5L was put into 10L ***** cultivation tank, inoculation of the above-mentioned preculture liquid was carried out, and it cultivated by 28 **, 300 rpm, and aeration 1vvm. Glucose concentration was maintained to 0.5 - 1.5 % by the feeding method, and it cultivated for ten days.

[0086] Gas chromatography analyzed the fatty acid methyl ester obtained by sampling a biomass temporally and carrying out methyl esterification like Example 1. Biomasses were collected after the end of culture and chloroform methanol extraction was performed after desiccation. Furthermore, when fractionation of the oil extracted in accordance with the conventional method was carried out by thin layer chromatography and silicic acid column chromatography, neutral lipid was [polar lipid] 8 % of the weight among the extracted oils 92% of the weight (91% of the weight which extracted triglyceride of an oil). Methyl esterification of the obtained triglyceride was carried out by the above-mentioned method, and gas chromatography analyzed it. Aging is shown in Table 6 and 7.

[0087]

[Table 6]

表 6

10L 容通気培養槽を用いた培養の経時変化

培養 日数 (日)	総脂肪酸に対する各脂肪酸の割合 (%)										ETA 生成量 (g/培地1L)
	16:0	18:0	18:1	18:2	18:3	8TA	ETeA	20:3	20:4	その他	
5	19.1	13.4	13.1	45.5	0.0	3.9	0.0	0.0	0.4	4.6	0.21
8	18.7	12.0	13.2	32.8	0.5	13.1	0.0	0.7	4.4	4.6	0.93
9	18.2	11.8	11.3	31.9	0.5	14.0	0.0	0.8	4.8	6.7	1.05
10	17.1	11.8	10.8	30.9	0.7	15.0	0.0	1.0	5.7	7.0	1.21

略号は表 1 と同じ。

[0088]

[Table 7]

表 7

トリグリセリドの脂肪酸組成の経時変化

培養 日数 (日)	トリグリセリド中の総脂肪酸に対する各脂肪酸の割合 (%)									
	16:0	18:0	18:1	18:2	18:3	ETA	ETeA	20:3	20:4	その他
5	20.8	13.9	13.7	44.5	0.0	3.0	0.0	0.0	0.2	3.9
8	19.7	13.0	13.8	32.2	0.3	12.1	0.0	0.5	4.1	4.3
9	19.2	12.8	12.2	31.0	0.3	13.4	0.0	0.6	4.5	6.0
10	18.0	12.7	11.5	30.9	0.6	14.0	0.0	0.8	5.0	6.5

略号は表 1 と同じ。

[0089]The mass culture method using 10L ***** cultivation tank showed that remarkable 5, 11, and 14-eicosatrienoic acid were producible. Remarkable 5, 11, and 14-eicosatrienoic acid were contained in the obtained triglyceride.

[0090]Preparation gelatin 100 of an example 7. capsule Water was added to the weight section and glycerin 35 weight section for foodstuffs addition, it dissolved at 50-60 **, and the gelatin coat with a viscosity of 20000 cps was prepared. Next, 3% of the weight of the vitamin-E oil was mixed for the hexane extraction oil or this hexane extraction oil which crushed the dried cell obtained by the method described in Example 6, and was extracted by hexane to deoxidation, deodorization, and the triglyceride produced by carrying out degumming processing, and contents were prepared. Using these, in accordance with the

conventional method, capsule molding and desiccation were performed and the soft capsule which contains the contents of 180 mg per grain was manufactured.

[Translation done.]